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Selective Extraction of Ephedrine from *Ephedra sinica* Using Mixtures of CO_2, Diethylamine, and Methanol

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Key Words

Supercritical fluid extraction
Basified modifier
Ephedrine derivatives
Ephedra sinica

Summary

The effect of modifiers has been investigated on the SFE efficiencies of ephedrine derivatives (e. g. methylephedrine, norephedrine, ephedrine, and pseudoephedrine) from aerial parts of *Ephedra sinica*. Among the modifiers employed, methanol containing 10 % (v/v) of diethylamine showed greater enhancement of SFE efficiency for ephedrine derivatives than any other modifiers evaluated in this study. These results might be due to the fact that the salts of *Ephedra* alkaloids (insoluble in CO_2) in plant tissues are changed to their free bases (freely soluble in CO_2) by basified modifiers such as diethylamine in methanol. It was supported by measurements of solubilities of these compounds in each supercritical solvent. In addition, CO_2 modified with diethylamine in methanol could more readily extract ephedrine than pseudoephedrine, which is the diastereomer of ephedrine.

Introduction

Numerous problems of conventional organic solvent extraction methods, i. e., high cost, toxicity, and environmental hazard, have promoted the wide use of supercritical CO_2 for extraction of natural products as an alternative [1–3]. Among the groups of natural products, alkaloids have drawn more attention as target compounds for supercritical fluid extraction (SFE) than any other because of their diverse biological activities. A number of SFE applications to the extraction of alkaloids including caffeine, monocrotaline, senecionine, seneciphylline, thebaine, codeine, morphine, *O*-methylcariachine, protopine, α-allocryptopine, escolzine, californidine, sanguinorine, and chelerythine from plant materials have been reported by previous researchers [4–10]. However, in the extraction of alkaloids using supercritical CO_2, there are some points to be considered; it is sufficiently nonpolar as not to extract or solubilize polar alkaloids. Moreover, most alkaloids do not exist as free bases (freely soluble in nonpolar solvent) but in salt forms (insoluble in nonpolar solvent) conjugated with several acids in plant tissues due to their characteristic basicities [11]. To overcome this deffiiciency of CO_2 for extraction of alkaloids, addition of a few modifiers which can increase the polarity of CO_2, or other supercritical fluids such as N_2O and CHF_3 have been utilized [12–14]. However, several problems with these alternative methods including limitations to the amount of added modifiers and environmental hazards of the alternative supercritical solvents have led to the development of other SFE methods before they became universally accepted as an extraction method for alkaloids.

In this study, we intended to extract ephedrine derivatives, benzylamine alkaloids, from aerial parts of *Ephedra sinica* using supercritical CO_2. The aerial parts of *Ephedra sinica* have long been used in traditional medicine as a diaphoretic, anti-asthmatic and diuretic as well as for the treatment of bronchitis and acute nephritic edema [15]. The alkaloids isolated from *E. sinica: Ephedra* alkaloids such as methylephedrine (**1**), norephedrine (**2**), ephedrine (**3**), pseudoephedrine (**4**) (Figure 1) have been used for asthma, influenza and some types of inflammation [15]. Unfortunately, in preliminary tests, pure CO_2 could not extract these ephedrine derivatives under any experimental conditions employed (40–80 °C, 13.6–34.0 MPa). Therefore, to enhance the SFE efficiency of ephedrine derivatives, we systematically carried out experiments as follows. (i) The solubilities (pure compound extractabilities or pseudosolubilities) of the free bases and the salts of ephedrine derivatives (hydrochloride salts in this study) were compared in order to evaluate the effect of the salt form on SFE efficiency. (ii) Methanol and water, as

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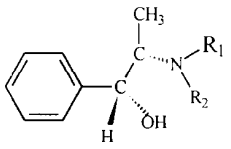
Table I. SFE experimental conditions for each sample.

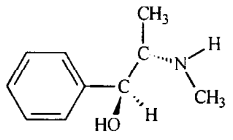
Sample	Temperature (°C)	Pressure (MPa)	Flow rate (mL min^{-1})	Static time (min)	Volume consumed (mL)
Pseudoephedrine free base	40 and 80	13.6–34.0	1.0	15	10
Salts of ephedrine derivatives	80	34.0	1.0	15	10
Spiked filter papers	80	34.0	1.0	15	50
Plant materials	80 and 100	34.0	1.0	15	50 and 100



R_1=R_2= CH_3; Methylephedrine (1)

R_1=R_2= H; Norephedrine (2)

R_1=CH_3, R_2= H; Ephedrine (3)



Pseudoephedrine (4)

Figure 1
Structures of methylephedrine (**1**), norephedrine (**2**), ephedrine (**3**), and pseudoephedrine (**4**).

initial modifiers, were used for improving the solubilities of ephedrine derivative salts. (iii) Next, the solubilities of the salts in non-basified modifiers such as pure methanol or water were compared with those of methanol or water basified with diethylamine. (iv) For measurements of desorption of ephedrine derivatives from a matrix by these solvents employed in this paper, the extractabilities from filter papers by the supercritical solvents were investigated. (v) Based upon the results of solubility determination and desorption from a matrix, SFE was performed for extraction of the alkaloids from a plant matrix (*E. sinica*).

Experimental

Plant Material

The aerial parts of *E. sinica* were from Korea Export and Import Federation of Drugs, Seoul, Korea. Their authenticity was confirmed by Dr. D. S. Han (Emeritus Professor, College of Pharmacy, Seoul, Korea). Plant materials were dried at 40 °C for 24 hrs and pulverized.

Chemicals and Standards

The HPLC grade methanol and water were from J. T. Baker Inc. (Phillipsburg, NJ, USA). Diethylamine (99 %) and diethylether (95 %) were from Duksan Chemical Co. (Yongin, Kyungki-Do, Korea). *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), orcinol, and pseudoephedrine free base were from Sigma Chemical Co. (St. Louis, MO, USA). Hydrochloride salts of methylephedrine, norephedrine, ephedrine, and pseudoephedrine were generously donated by Dr B.-J. Cha (Dong-A Pharm, Co. Ltd., Anyang, Kyungki-Do, Korea).

Spiking Ephedrine Derivatives onto Filter Paper

1 g filter paper disks (Advantec No. 2, Toyo Roshi Kaisha, Japan) were cut to *ca.* 1 cm diameter and placed in the extraction vessel. Each ephedrine derivative standard (0.2 mg) was spiked onto the filter paper disks. They were then dried in vacuum oven at 40 °C for 24 hours.

Organic Solvent Extraction

The dried and pulverized plant materials (1.0 g) were extracted with 70 mL 0.5 M H_2SO_4 for 12 h [16]. The filtered extract was adjusted to pH 11–13 by addition of 6 M NaOH. 16 g NaCl was added in the extract to remove the salts and extracted with 100 mL diethyl ether three times. The diethyl ether phase was evaporated to dryness and the residue dissolved in 10 mL methanol. From this, 1 mL methanol solution was re-evaporated together with 50 μg orcinol as an internal standard for GC analysis.

Supercritical Fluid Extraction

SFE was on an Isco supercritical fluid extractor, model SFX 3560 equipped with two Isco 260 D syringe pumps (Lincoln, NE, U.S.A.) using CO_2 (99.9 %, Seoul Gas Co. Seoul, Korea) and CO_2 modified with 1, 5 and 10 % of methanol, water, methanol containing 10 % (v/v) of diethylamine, and water containing 10 % (v/v) diethylamine. The SFE experimental conditions are listed in Table I. The restrictor temperature was the same at each extraction temperature. The remaining volume was filled with glass wool. In each extraction step, the extract was collected in methanol.

GC Analysis

For measurement of the solubility of pseudoephedrine free base, the extracts in methanol were evaporated un-

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der reduced pressure and dissolved in an appropriate amount of chloroform (1–10 mL). Then, 1 mL of the chloroform solution was re-evaporated together with 1 mg palmitic acid methyl ester as an internal standard under a N_2 stream, and dissolved in 1 mL chloroform for GC analysis. SFE extracts of hydrochloride salts of ephedrine derivatives, spiked filter papers, and plant materials were dissolved in 1 mL methanol and transferred into a reaction vial. Orcinol (50 μg) was used as an internal standard and added to each solution and evaporated under N_2 stream. Extracts were TMS-derivatized with 100 μL MSTFA at 65 °C for 90 min. GC-FID was on a Hewlett Packard (Avondale, PA, U.S.A.) 5890 series II gas chromatograph equipped with HP 3395 integrator and capillary GC column (Ultra 1, crosslinked methylsiloxane, 25 m × 0.32 mm, film 0.52 μm, HP). Helium was used as carrier gas at 3.7 mL min^{-1}. The split ratio was 20:1. The oven temperature was increased from 90 (1 min hold) to 124 °C at 3 °C min^{-1} (3 min hold) and then to 280 °C at 20 °C min^{-1}. The injector and detector temperatures were 220 °C and 280 °C, respectively. The GC chromatogram of TMS standards of methylephedrine (**1**), norephedrine (**2**), ephedrine (**3**), and pseudoephedrine (**4**) is in Figure 2.

Results and Discussion

Solubilities of Free Base and Salt of Pseudoephedrine in Supercritical CO_2

Ephedrine derivatives were not extracted at all from the aerial parts of *E. sinica* by pure supercritical CO_2 in the preliminary tests. This may be due to the fact that most alkaloids exist as their salt forms in plant tissues because of their own characteristic basicity [11]. Hence the solubilities of these salts should be compared with those of free bases prior to conducting SFE. For this purpose, the solubilities of pseudoephedrine free base and its salts in supercritical CO_2 were investigated at varying temperatures (40 and 80 °C) and pressures (13.6–34.0 MPa), and then compared. In the present investigation, the effects of static time and flow rate for equilibrium between solute and solvent were not evaluated since the purpose of this work was to measure only the effects of various modifiers on extractabilities of target compounds. Therefore, although the term "solubility" is used here, it may be "pure compound extractability" or "pseudosolubility" in the strict sense, rather than the true solubility. Solubilities of pseudoephedrine free base in supercritical CO_2 under the conditions employed are in Figure 3. The solubility increased with increasing pressure at 80 °C, while it did not show any significant enhancement at 40 °C. The solubility of pseudoephedrine in supercritical CO_2 at 80 °C and 34.0 MPa, in which its solubility had the highest value, was determined as 2.47 (± 0.22) mg mL^{-1}. In contrast with these results, however, supercritical CO_2 could not extract pseudoephedrine hydrochloride salt under any experimental conditions. These results proved that pure supercritical CO_2 was unsuitable for extraction of salt forms of ephedrine derivatives in plant tissue. Thus, we intended to use the modifier enhancing the polarity of CO_2 in order to increase solubilities of ephedrine derivative salts.

Effect of Methanol and Water on the Solubility of Ephedrine Derivative Salts

To improve solubilities of ephedrine derivative hydrochloride salts, methanol or water as modifier was introduced into CO_2 at 80 °C and 34.0 MPa by separating syringe pump at concentrations of 1, 5 and 10 % (v/v), respectively. The effect of methanol and water is shown in Figure 4. Water was only efficient for methylephedrine hydrochloride salt but it had little effect on the others. The inefficiency of water may be originated from its low miscibility with CO_2. Previous reports showed that the solubility of water in CO_2 was only 0.5 % (v/v) at 75 °C and 34.0 MPa [17] hence the large volume of water added may cause phase separation or aerosol formation with CO_2. In the case of methanol, however, significant improvements in the solubilities of ephedrine derivative salts employed in this study relative to water were revealed. Even though the addition of methanol in CO_2 resulted in slight improvements in solubilities, they were still poor; hence another modifier to enhance the solubilities of ephedrine derivative salts was required.

Effect of Modifiers Basified with Diethylamine on Solubilities of Ephedrine Derivative Hydrochloride Salts

Generally, alkaloid salts are insoluble in nonpolar solvents but their free bases are quite soluble in them. Therefore, to solubilize alkaloids in CO_2, the basified modifier should be introduced into the SFE. For this purpose, diethylamine in water or methanol (10 % v/v) was eluted at concentrations of 1, 5, and 10 % (v/v). Figure 5 shows the solubilities of ephedrine derivative hydrochloride salts in CO_2 mixed with each basified modifier. Except for methyl ephedrine hydrochloride, on which the basified modifiers had little enhancing effect relative to neat methanol or water, the addition of modifiers basified with diethylamine greatly increased solubilities when compared to pure methanol or water. Amine modifiers have been employed in packed column SFC to improve peak shapes and chromatographic efficiency for basic solutes [18–20]. In SFE, 1,6-hexanediamine as modifier has been used for the extraction of amine analytes from soil [21] and triethylamine for cocaine from hair [22]. As these previous reports show, the basified modifiers showed a larger increase in the solubilities of ephedrine derivative salts compared to neutral ones. The mixtures of CO_2-methanol-diethylamine (90:9:1) in particular extracted these compounds more effectively than all those employed, where the solubilities were 213–564 μg mL^{-1}.

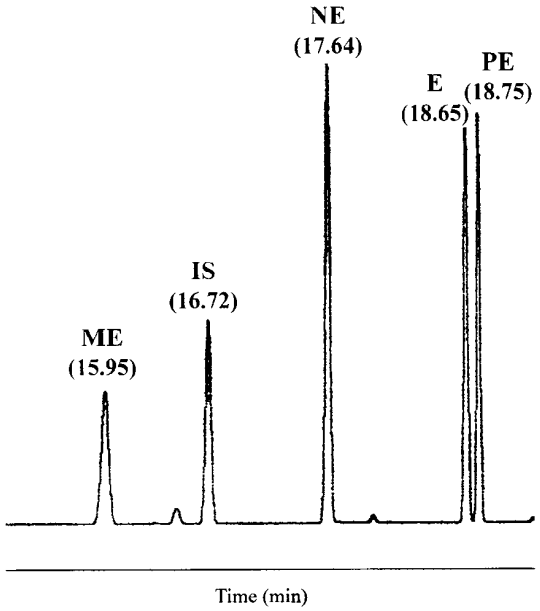

Figure 2

GC chromatogram of ephedrine derivatives. ME = methylephedrine (**1**); NE = norephedrine (**2**); E = ephedrine (**3**); PE = pseudoephedrine (**4**); IS = internal standard.

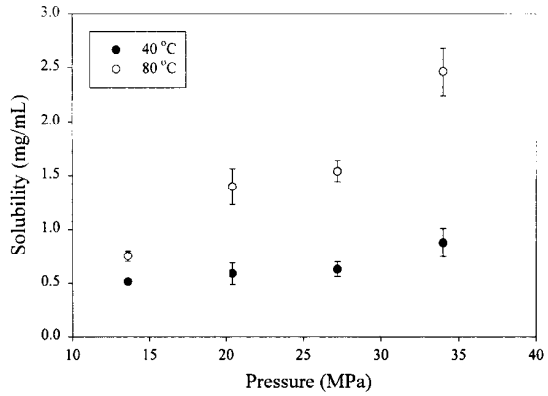

Figure 3

Solubility of pseudoephedrine free base in supercritical CO_2 at 40 and 80 °C in range 13.6–34.0 MPa. All experiments in triplicate

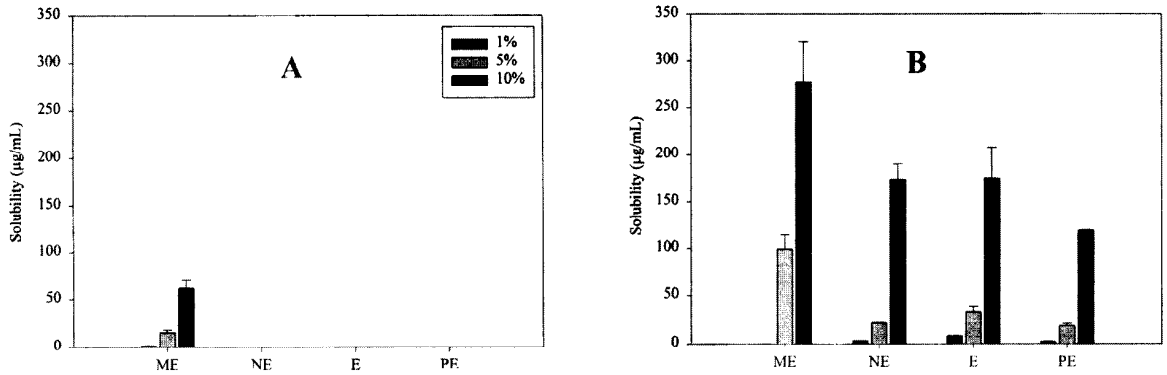

Figure 4

Effect of water (**A**) and methanol (**B**) modifier on solubilities of ephedrine derivative hydrochloride salts in CO_2 at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10 %, respectively. ME = methylephedrine; NE = norephedrine; E = ephedrine; PE = pseudoephedrine. All experiments in triplicate

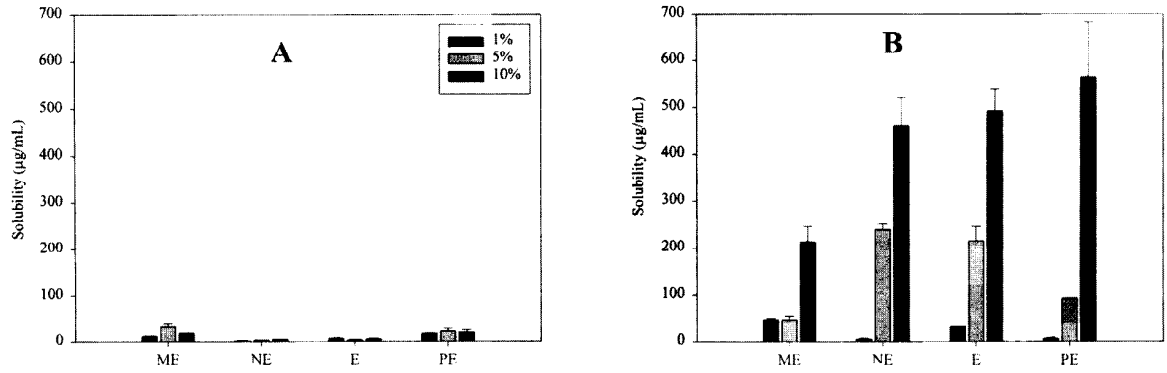

Figure 5

Effect of water (**A**) and methanol (**B**) basified with diethylamine (10 % v/v) as modifier on solubilities of ephedrine derivative hydrochloride salts in CO_2 at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10 %, respectively. ME = methylephedrine; NE = norephedrine; E = ephedrine; PE = pseudoephedrine. All experiments in triplicate*

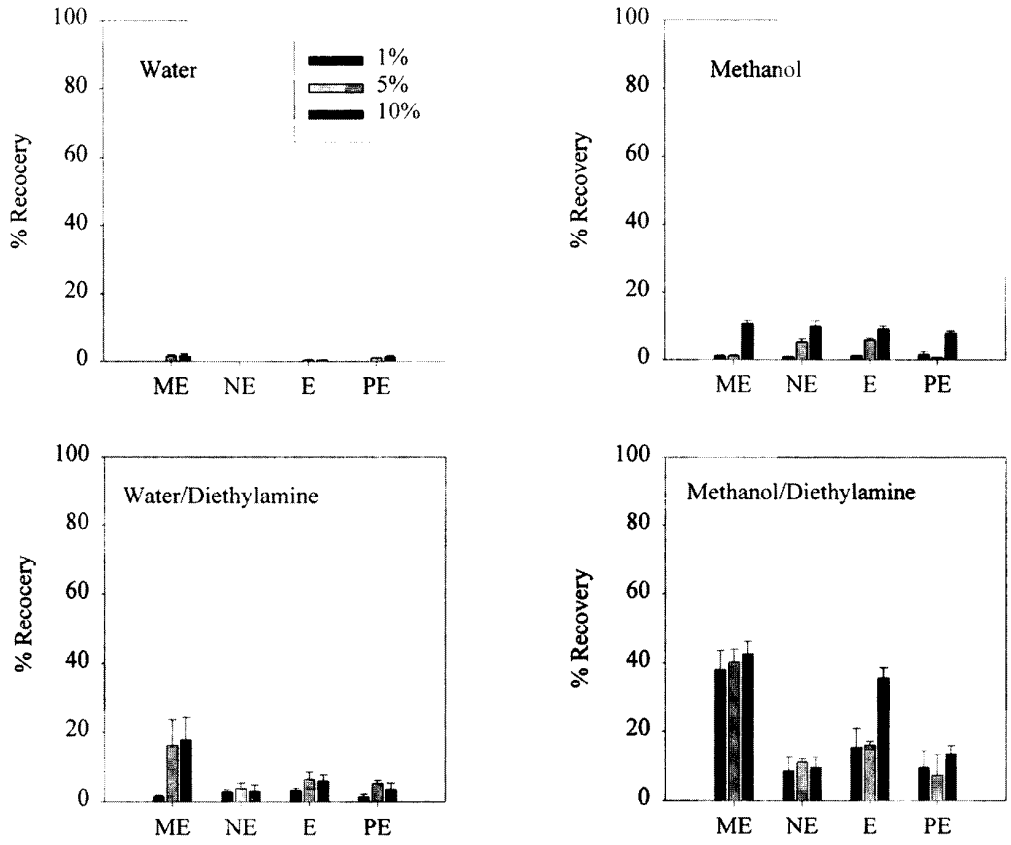

Figure 6
Effect of modifiers on recovery of ephedrine derivatives from filter papers at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10%, respectively. ME = methylephedrine; NE = norephedrine; E = ephedrine; PE = pseudoephedrine. All experiments in triplicate

Effect of Modifiers on Desorption from Matrices

In addition to the effect of modifiers on solvating power, they also play other very important roles, especially for matrices, where the analyte is strongly bound through chemisorption and physisorption. Another advantage from using a polar modifier is swelling of the matrix, thereby increasing the internal volume, which in turn increases the surface area accessible to the near supercritical solvents [23, 24]. Therefore, prior to SFE, the effect of modifiers on a matrix should be evaluated together with that on solubility. These results may demonstrate that specific interactions occur between ephedrine derivatives and a matrix. In this step, the effects of modifiers were investigated on the SFE extractabilities of ephedrine derivatives from filter papers. Figure 6 shows the effect of modifiers on the recoveries of ephedrine derivative hydrochloride salts from filter papers. In these results, diethylamine in water or methanol was more efficient in the extraction of ephedrine derivatives from filter papers than pure water and methanol as shown by solubility measurement. In particular, diethylamine in methanol could extract more than twice as much of these compounds as any other modifiers tested in this study. Therefore, these results suggest that diethylamine in methanol is the most effective in improving SFE efficiencies of ephedrine derivatives by improving desorption from matrices as well as enhancing solubilities.

SFE for Ephedrine Derivatives from *E. sinica*

In the test of solubility and desorption from filter papers, diethylamine in methanol as a modifier was found to be best for extraction of ephedrine derivatives. Figure 7 shows the effect of modifiers on SFE efficiencies from plant materials. Diethylamine in methanol was obviously effective for SFE efficiencies of all the ephedrine derivatives from plant materials as shown by the results of solubility and desorption from filter papers. In particular, 80 % methylephedrine could be extracted using 10 % of this modifier compared to conventional organic solvent extraction. Although diethylamine in methanol was most effective among the modifiers for improving SFE efficiencies of ephedrine derivatives from plant materials, the yields of norephedrine, ephedrine, and pseudoephedrine were still 60 % relative to

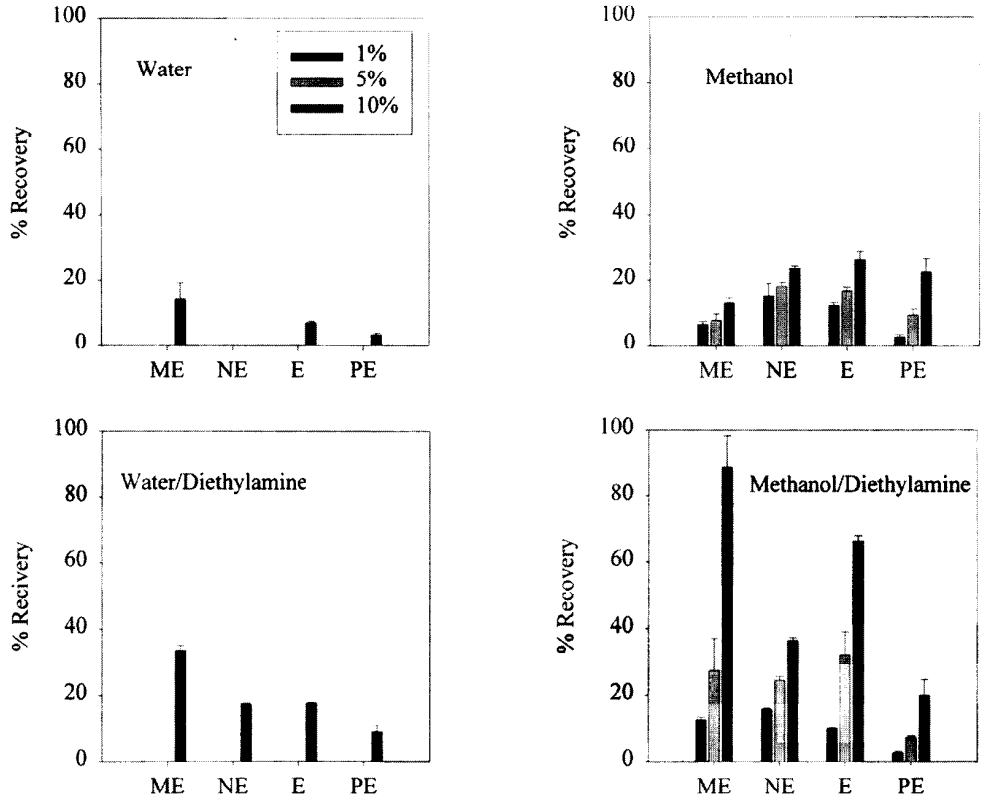

Figure 7

Effect of modifiers on recovery of ephedrine derivatives from aerial parts of *E. sinica* at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10%, respectively. ME = methylephedrine; NE = norephedrine; E = ephedrine; PE = pseudoephedrine. All experiments in triplicate

liquid extraction hence methods to enhance SFE yields of ephedrine derivatives should be performed. For this purpose, increase in extraction time, temperature, and proportion of modifier were carried out. Unfortunately, extraction time and temperature did not show any significant effects. However, when the amount of diethylamine in methanol was increased up to 20%, extraction yields of ephedrine derivatives were dramatically improved. Methylephedrine and ephedrine were completely recovered by SFE using this modifier. Yields of ephedrine derivatives by CO_2 modified with 20 % diethylamine in methanol were compared with those by conventional extraction in Table II. In addition to enhancement of yields, an important result was obtained by this method. Pseudoephedrine, the diastereomer (Figure 1), only 44 % was extracted, while the ephedrine ever was 106 %. It may not be caused by differences in their solubilities but by desorption from plant matrix. As shown in Figures 4 and 5, the yield of pseudoephedrine extracted by CO_2 modified with diethylamine in methanol from filter papers was about half of that of ephedrine, while there was no significant difference in their solubilities. The GC chromatogram obtained by SFE and liquid extraction is in Figure 8. These results suggest the probability that SFE can extract target compounds selectively from its stereoisomers.

Conclusion

This paper investigates the effect of modifiers on extraction yields of ephedrine derivatives. Among modifiers employed, diethylamine in methanol was most effective. The mechanism of this modifier in enhancing the SFE yields of compounds from plant materials was supported by increased solubilities in CO_2 and desorption from filter papers. The improvement of solubilities was found to be caused by changing salts of ephedrine derivatives (insoluble in CO_2) to free bases (freely soluble in CO_2). In addition to increasing solubilities, diethylamine in methanol as a modifier could greatly improve desorption of ephedrine derivatives from a matrix. Another important results; CO_2 modified with diethylamine in methanol could extract ephedrine more selectively than pseudoephedrine, the diastereomer of ephedrine

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Table II. Yields of methylephedrine (ME), norephedrine (NE), ephedrine (E), and pseudoephedrine (PE) obtained by organic solvent extraction[1] and SFE[2]. Results are mg g^{-1} (% RSD).

Extraction method	ME	NE	E	PE
Organic solvent extraction	0.25 (1.2)	0.12 (16.9)	3.22 (6.0)	1.1 (6.0)
SFE	0.37 (0.4)	0.046 (25.1)	3.44 (2.2)	0.40 (8.4)

All experiments in triplicate

[1] extraction solvent 0.5 M H_2SO_4 followed by basifying with 6M NaOH and diethylether extraction.

[2] mixture CO_2-methanol-diethylamine (80:18:2). temperature and pressure 80 °C and 34.0 MPa, respectively. static time 15 min. total amount of solvent cosumed 50 mL during dynamic extraction.

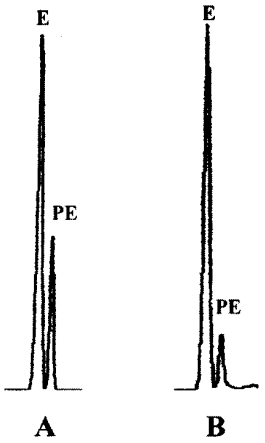

Figure 8
Comparison of GC chromatogram obtained by 0.5 M H_2SO_4 extraction (**A**) and SFE at 80 °C and 34.0 MPa using 20 % diethylamine in methanol modifier (**B**). E = ephedrine, PE = pseudoephedrine.

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